Forensic Science International 245 (2014) 65–71

Contents lists available at ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

Hydrocarbon profiles throughout adult Calliphoridae aging: A promising tool for forensic entomology

Jennifer L. Pechal [a,*], Hannah Moore [b], Falko Drijfhout [b], M. Eric Benbow [c,*]

[a] Department of Entomology, 243 Natural Science, Michigan State University, East Lansing, MI 48824, USA
[b] School of Physical and Geographical Sciences, Keele University, ST5 5BG Staffordshire, UK
[c] Department of Entomology and Department of Osteopathic Medical Specialties, 243 Natural Science, Michigan State University, East Lansing, MI 48824, USA

ARTICLE INFO

Article history:
Received 18 July 2014
Received in revised form 2 October 2014
Accepted 3 October 2014
Available online 14 October 2014

Keywords:
Forensic entomology
Calliphoridae
Hydrocarbons
Adult aging

ABSTRACT

Blow flies (Diptera: Calliphoridae) are typically the first insects to arrive at human remains and carrion. Predictable succession patterns and known larval development of necrophagous insects on vertebrate remains can assist a forensic entomologist with estimates of a minimum post-mortem interval (PMI_{min}) range. However, adult blow flies are infrequently used to estimate the PMI_{min}, but rather are used for a confirmation of larval species identification. Cuticular hydrocarbons have demonstrated potential for estimating adult blow fly age, as hydrocarbons are present throughout blow fly development, from egg to adult, and are stable structures. The goal of this study was to identify hydrocarbon profiles associated with the adults of a North American native blow fly species, *Cochliomyia macellaria* (Fabricius) and a North American invasive species, *Chrysomya rufifacies* (Macquart). Flies were reared at a constant temperature (25 °C), a photoperiod of 14:10 (L:D) (h), and were provided water, sugar and powdered milk *ad libitum*. Ten adult females from each species were collected at day 1, 5, 10, 20, and 30 post-emergence. Hydrocarbon compounds were extracted and then identified using gas chromatography–mass spectrometry (GC–MS) analysis. A total of 37 and 35 compounds were detected from *C. macellaria* and *Ch. rufifacies*, respectively. There were 24 and 23 *n*-alkene and methyl-branched alkane hydrocarbons from *C. macellaria* and *Ch. rufifacies*, respectively (10 compounds were shared between species), used for statistical analysis. Non-metric multidimensional scaling analysis and permutational multivariate analysis of variance were used to analyze the hydrocarbon profiles with significant differences ($P < 0.001$) detected among post-emergence age cohorts for each species, and unique hydrocarbon profiles detected as each adult blow fly species aged. This work provides empirical data that serve as a foundation for future research into improving PMI_{min} estimates made by forensic practitioners and potentially increase the use of adult insects during death investigations.

1. Introduction

Blow fly (Diptera: Calliphoridae) adults are primary colonizers of decomposing vertebrate remains (e.g., swine carcasses and human cadavers) and are commonly the first forensically important insect taxa to arrive at cadavers post-mortem. Forensic entomologists can use the predictable succession patterns, as determined by adult insect arrival (e.g., flies then beetles) and colonization patterns, along with the subsequent dipteran larval development to estimate a minimum post-mortem interval (PMI_{min}) range. Typically, forensic entomologists receive entomological evidence collected from a death investigation scene and compare the developmental stage (e.g., third instar or pupa of a dipteran species) of the specimen to its known life history information [1–3]. Using this information an expert can estimate the age range of the insect, which can be used as a surrogate for PMI_{min} estimations. However, adult blow flies are infrequently used by practitioners for PMI_{min} estimations, as there is no established standard for estimating adult age and adult Calliphoridae specimens are primarily used for a confirmation of larval and pupal identifications.

Recently, cuticular hydrocarbons have demonstrated potential for estimating adult blow fly age [4,5], which has importance in forensic applications, especially when there are no larval dipteran specimens present at a crime scene. While there have been several methods, such as assessing pteridine levels in the eye [6], counting cuticular bands [7], and evaluating female ovarian status [8,9], used to estimate adult blow fly age; hydrocarbons could be reliable

* Corresponding authors. Tel.: +1 517 355 6514; fax: +1 517 432 7061.
E-mail addresses: pechalje@msu.edu (J.L. Pechal), hanamoore@yahoo.co.uk (H. Moore), f.drijfhout@keele.ac.uk (F. Drijfhout), benbow@msu.edu (M.E. Benbow).

http://dx.doi.org/10.1016/j.forsciint.2014.10.019
0379-0738/

indicators of insect age, as these are stable structures present throughout blow fly development (i.e., from egg to adult). An insect's cuticle is covered by a layer of hydrocarbons comprised of *n*-alkanes, *n*-alkenes, and terminally and internally branched alkanes. The function of these hydrocarbons are to prevent desiccation [10]; reduce external attack by toxins such as insecticides, bacteria and fungal pathogens [11,12]; and function as pheromones or kairomones [10,13], which can be important in regulating adult insect behavior, such as mate selection and oviposition events.

Previous work identifying hydrocarbon compounds in Calliphoridae has resulted in detecting specific hydrocarbon profiles for individual taxon, along with specific profiles resulting from sex and developmental stage differences within each taxon. For example, hydrocarbon profiles have been used to differentiate the pupae of six necrophagous flies [14], which is important because taxonomical keys for pupae are not as complete as other life history stages (e.g., adults and third instar larvae), and pupae or empty puparia are often collected as entomological evidence at indoor death investigations. Distinct hydrocarbon profiles have also been detected in different life history stages within Calliphoridae: *Chrysomya rufifacies* (Macquart) larvae [5], *Lucilia sericata* (Meigen) larvae [15], and *Ch. megacephala* (F.) pupae [16]. Furthermore, hydrocarbon profile changes throughout development have been identified in *Lucilia cuprina* Wiedemann puparia and adults [17]; and all developmental stages from eggs to 8-day adults for *Calliphora vicina* Robineau-Desvoidy, *Ca. vomitoria* L. and *Protophormia terraenovae* Robineau-Desvoidy [18]. There have also been distinct hydrocarbon differences reported between male and female *Ch. bezziana* (Villeneuve) [19], and differences in *Ca. vomitoria* adult hydrocarbon composition and abundance with alkenes only present in females [20]. Further, hydrocarbon complexity was described for females as they progressed through the vitellogenic cycle [21]. Additionally, cuticular hydrocarbons have demonstrated promise for identifying geographic regions of origin based on adult profiles [22]. Many of these studies have direct implications for use by a forensic entomologist to improve the age estimation of an individual insect specimen, as they provide comprehensive lists of hydrocarbon profiles present during the development of several calliphorid taxa. However, the implications and applicability of estimating a PMI_{min} range based on insect hydrocarbon profiles (e.g., predictive modeling) in forensics sciences are only beginning to be thoroughly explored.

Blow flies are spatially and temporally distributed throughout North America and the world [23]. *Cochliomyia macellaria* (Fabricius) is considered a primary colonizer of vertebrate remains [24,25], while *Ch. rufifacies* on the other hand is a secondary colonizer [26] and typically utilizes the vertebrate resource during more advanced progression of the decomposition process [27,28]. *Chrysomya rufifacies* is an invasive species that was introduced into the United States in the 1980s [29]; it has expanded its distribution over the past 25 years and has been collected as far north in lower parts of Canada [30]. This species is a facultative predator and will consume larvae of prior dipteran colonizers or cannibalize conspecific larvae [28,31]. The hairy blow fly larvae, *Ch. rufifacies*, can be distinguished from other dipteran larvae on a resource by the spine-like projects on each segment [23].

The goal of this study was to identify hydrocarbon profiles associated with the adults of a North American native blow fly species, *C. macellaria*, and an invasive species, *Ch. rufifacies*, at discrete time intervals post-emergence (1, 5, 10, 20 and 30 days) to characterize age-associated hydrocarbon profiles throughout adult female development.

2. Materials and methods

Post-feeding third instar larvae and pupae were collected from a decomposing swine (*Sus scrofa* L.) carcass in July 2012 and resulting adults were identified using a key for Calliphoridae adults north of Mexico [23]. *Cochliomyia macellaria* and *Ch. rufifacies* adults (F_0 generation) were reared separately at a constant temperature (25 °C), a photoperiod of 14:10 (L:D) (h), and provided water, sugar and powdered milk *ad libitum*. Ten adult females from each species (F_1 generation) were collected on day 1, 5, 10, 20, and 30 post-emergence and immediately stored individually in vials at 4 °C until hydrocarbons were extracted. Individual females from the five age cohorts were immersed in 400 μl *n*-hexane for 15 min at room temperature (25 °C) to obtain hydrocarbon compounds. Females were the predominant sex of adult individuals collected at the carcass (results not shown), and thus were the focus for the hydrocarbon profile assessment.

Chemical analysis of all extracts was carried out on an Agilent Technologies 6890N Network GC with a split/splitless injector at 250 °C, a Restek Rxi-1MS capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) and coupled to an Agilent 5973 Network Mass Selective Detector. The GC was coupled to a computer and data processed with Agilent Chemstation software. Elution was carried out with helium at 1 mL/min. The oven temperature was programmed to be held at 50 °C for 2 min then ramped to 200 °C at 25 °C/min, then from 200 to 260 °C at 3 °C/min and finally from 260 to 320 °C at 20 °C/min, where it was held for 2 min. The mass spectrometer was operated in electron ionisation mode at 70 eV, scanning from 40 to 500 amu at 1.5 scans s^{-1}. Hydrocarbons were identified using a library search (NIST08), the diagnostic fragment ions and the Kovats indices.

2.1. Statistical analyses

Chromatographic peak areas of alkenes and methyl branched alkane compounds (*n*-alkanes excluded) were used for statistical analyses. Peak area relative abundance was arc-sine square root transformed prior to subsequent multivariate analyses. Analyses were performed using the vegan 2.0-9 library in the R statistical package [32,33]. Permutational multivariate analysis of variance (PERMANOVA) tested for adult hydrocarbon profile differences among days post-emergence using the adoins function; PERMANOVA is a non-parametric technique used to differentiate groups (e.g., days post-emergence) based on a Bray-Curtis dissimilarity matrix [33]. The Bray-Curtis distance was also used for non-metric multidimensional scaling analysis (NMDS), which is a non-parametric ordination technique that avoids assuming linearity among community variables [34]. Differences in hydrocarbon profiles were further visualized with heatmaps using the gplots 2.12.1 library in the R statistical package; dendrograms were clustered on the top and side by similarity in values.

3. Results

The *C. macellaria* profile consisted of 37 compounds detected from adults aged day 1 to 30 post-emergence: the hydrocarbon profile consisted of *n*-alkanes (27%), *n*-alkenes (3%) and methyl-branched alkanes (70%) with chain lengths ranging from C23 to C33. The profile of *Ch. rufifacies* consisted of 35 compounds with *n*-alkanes (29%), *n*-alkenes (17%) and methyl-branched alkanes (54%) detected from day 1 to 30 post-emergence; these chain lengths ranged from C21 to C33. Of the 37 and 35 resolvable peaks extracted from the cuticle of both adult blow fly species (data not shown), 24 and 23 *n*-alkene and methyl-branched alkane peaks were used in the subsequent statistical analysis; the mean (±standard deviation) composition of hydrocarbons compounds varied as both *C. macellaria* and *Ch. rufifacies* female adults aged (Tables 1 and 2).

Although the profiles of the two species shared ten compounds, both displayed very unique hydrocarbon profiles (Tables 1 and 2). The hydrocarbon profiles of each species shifted as the adult

Table 1
The mean (±SD) relative abundance of methyl-branched alkane hydrocarbons for female *Cochliomyia macellaria* adults at day 1, 5, 10, 20 and 30 post-emergence.

Peak no.	Hydrocarbon	Kovats	Day 1	Day 5	Day 10	Day 20	Day 30
1	11 + 13-MeC25	2536	9.09 ± 7.04	13.73 ± 4.55	10.39 ± 4.47	10.40 ± 4.99	8.05 ± 3.19
2	5-MeC25*	2554	tr	1.78 ± 0.50	1.49 ± 0.66	1.39 ± 0.56	0.90 ± 0.33
3	9,13-DiMeC25*	2569	1.33 ± 0.94	1.98 ± 0.75	1.20 ± 0.51	1.49 ± 0.85	1.66 ± 0.77
4	3-MeC25	2574	2.18 ± 2.47	7.18 ± 2.66	6.26 ± 3.25	5.65 ± 2.63	3.89 ± 1.64
5	5,15-DiMeC25*	2586	tr	1.18 ± 0.33	0.89 ± 0.33	1.04 ± 0.51	0.82 ± 0.31
6	3,x-DiMeC27*	2609	tr	1.91 ± 0.66	1.61 ± 0.64	1.94 ± 0.99	1.82 ± 0.75
7	12,14-DiMeC26*	2635	tr	2.80 ± 0.76	2.43 ± 0.82	1.97 ± 0.86	1.81 ± 0.64
8	2-MeC26	2662	4.39 ± 2.62	2.74 ± 1.17	2.01 ± 1.09	1.10 ± 0.49	0.93 ± 0.54
9	9 + 11 + 13 + 15-MeC27	2732	8.21 ± 7.51	15.76 ± 4.74	5.16 ± 1.89	16.33 ± 8.77	21.30 ± 10.10
10	7-MeC27	2745	tr	5.07 ± 1.69	6.50 ± 2.84	5.09 ± 2.58	6.51 ± 3.00
11	5-MeC27	2754	tr	4.58 ± 1.65	3.71 ± 1.40	4.71 ± 2.36	4.86 ± 2.16
12	9,13-DiMeC27*	2763	2.41 ± 1.71	2.84 ± 1.25	2.12 ± 0.80	3.46 ± 1.75	5.81 ± 2.74
13	3-MeC27	2773	7.07 ± 3.12	24.56 ± 10.56	39.45 ± 20.68	36.61 ± 20.23	28.80 ± 23.34
14	5,x-DiMeC27*	2786	tr	1.49 ± 0.44	1.66 ± 0.78	1.91 ± 1.01	1.84 ± 1.29
15	3,7-DiMeC27*	2813	tr	2.46 ± 0.84	1.05 ± 0.47	3.55 ± 1.77	4.54 ± 2.21
16	12,14-DiMeC28*	2836	tr	0.82 ± 0.27	2.70 ± 1.21	0.83 ± 0.41	1.13 ± 0.52
17	2-MeC28	2870	21.68 ± 9.83	4.00 ± 1.88	8.07 ± 4.86	0.94 ± 0.43	1.01 ± 0.71
18	9 + 11 + 13 + 15-MeC29	2938	10.26 ± 3.37	1.80 ± 0.67	1.29 ± 0.79	1.59 ± 0.71	2.34 ± 1.78
19	7-MeC29	2948	2.49 ± 0.76	tr	1.02 ± 0.63	tr	0.98 ± 0.82
20	5-MeC29	2958	2.00 ± 0.63	tr	tr	tr	tr
21	x,y-DiMeC29*	2968	3.83 ± 1.39	tr	tr	tr	tr
22	3-MeC29	2979	5.60 ± 2.04	1.07 ± 0.49	0.97 ± 0.59	tr	1.00 ± 0.60
23	2-MeC30	3068	11.36 ± 4.99	1.79 ± 1.11	tr	tr	tr
24	x-MeC31**	3115	8.09 ± 2.38	0.46 ± 0.28	tr	tr	tr

tr = Detected in trace amounts (<0.5%).
* Tentative identification based on Kovats Index value and match with NIST08 Library database.
** Methyl branch position not determined.

females aged (Figs. 1 and 2), thus indicating there are unique profiles as adult aging occurs. We calculated the number of hydrocarbon compounds as identified by individual peaks and described this as hydrocarbon richness; this is analogous to species richness in ecological studies that provides the number of species in a given sample. Hydrocarbon richness was greater in *C. macellaria* than in *Ch. rufifacies* on all days except for the first day following emergence. Hydrocarbon richness plateaued after day 5 for *C. macellaria* with 16, 22, 19, 21 and 21 compounds detected on day 1, 5, 10, 20 and 30 days post-emergence, respectively. While, *Ch. rufifacies* adult female hydrocarbon richness demonstrated a decrease prior to an increase with 18, 17, 11, 16 and 15 compounds detected on day 1, 5, 10, 20 and 30 days post-emergence, respectively.

There were significant cuticular hydrocarbon profile differences ($P < 0.001$) among sampling days post-emergence for each species (Table A1). A two-dimensional NMDS ordination explained 99.6% (stress = 0.048) of hydrocarbon structure for *C. macellaria* females (Fig. 3). The first day post-emergence comprised of a distinctly different hydrocarbon profile compared with all of the other days, as indicated by the heatmap (Fig. 3). Hydrocarbon profiles from *C. macellaria* clustered individually by age cohort on day 1, 5 and 10,

Table 2
The mean (±SD) relative abundance of *n*-alkene and methyl-branched alkane hydrocarbons for female *Chrysomya rufifacies* adults at day 1, 5, 10, 20 and 30 post-emergence.

Peak no.	Hydrocarbon	Kovats	Day 1	Day 5	Day 10	Day 20	Day 30
1	C22 Alkadiene	2404	tr	tr	tr	3.05 ± 2.12	1.39 ± 0.69
2	2-MeC26	2665	tr	tr	0.83 ± 0.50	0.97 ± 0.66	0.79 ± 0.39
3	11 + 15-MeC27	2736	tr	3.01 ± 2.51	6.75 ± 4.94	6.43 ± 4.19	6.68 ± 3.31
4	9,13-DiMeC27*	2765	tr	tr	tr	0.46 ± 0.38	tr
5	3-MeC27	2775	1.70 ± 0.87	0.95 ± 0.67	tr	0.63 ± 0.40	tr
6	2-MeC28	2871	9.98 ± 5.53	10.23 ± 9.87	12.67 ± 7.04	12.85 ± 9.89	12.45 ± 6.60
7	C29 Alkene	2878	tr	5.16 ± 3.79	21.15 ± 18.66	17.62 ± 12.18	21.95 ± 16.69
8	13 + 15-MeC29	2938	2.17 ± 1.15	3.05 ± 2.60	2.88 ± 1.60	3.55 ± 2.63	2.66 ± 1.16
9	7-MeC29	2949	0.78 ± 0.31	0.34 ± 0.23	tr	tr	tr
10	5-MeC29	2958	0.69 ± 0.25	tr	tr	tr	tr
11	x,y-DiMeC29**	2969	0.70 ± 0.27	0.53 ± 0.46	tr	tr	tr
12	3-MeC29	2979	2.76 ± 1.03	1.39 ± 1.04	1.14 ± 0.87	1.07 ± 0.67	0.78 ± 0.33
13	12 + 14-MeC30	3036	1.81 ± 0.75	1.02 ± 0.95	tr	1.02 ± 0.77	tr
14	C31 Alkadiene	3048	tr	tr	2.66 ± 1.62	tr	2.85 ± 1.48
15	2-MeC30	3072	47.71 ± 21.12	36.56 ± 38.24	17.28 ± 12.55	17.67 ± 17.79	13.87 ± 8.93
16	C31 Alkene	3079	5.11 ± 1.63	21.65 ± 17.54	26.11 ± 22.08	26.02 ± 21.94	27.99 ± 23.90
17	11 + 13 + 15-MeC31	3130	17.83 ± 5.02	12.01 ± 10.57	6.30 ± 3.95	7.25 ± 5.36	6.24 ± 2.80
18	12 + 14-MeC32	3226	2.13 ± 1.11	1.36 ± 0.92	tr	0.90 ± 0.67	tr
19	14,16-DiMeC32*	3257	1.98 ± 0.60	tr	tr	0.49 ± 0.27	tr
20	C33 Alkadiene	3264	tr	1.80 ± 1.00	2.23 ± 1.40	tr	2.22 ± 0.98
21	2-MeC32	3271	1.57 ± 0.58	0.94 ± 0.61	tr	tr	0.14 ± 0.19
22	DiMe**	N/A***	2.08 ± 0.59	tr	tr	tr	tr
23	11 + 13 + 15 + 17-MeC33	N/A***	1.02 ± 0.42	tr	tr	tr	tr

tr = Detected in trace amounts (<1%).
* Tentative identification based on Kovats Index value and match with NIST08 Library database.
** Methyl branch position not determined.
*** Retention time for *n*-C34 not available, so could not obtain accurate value.

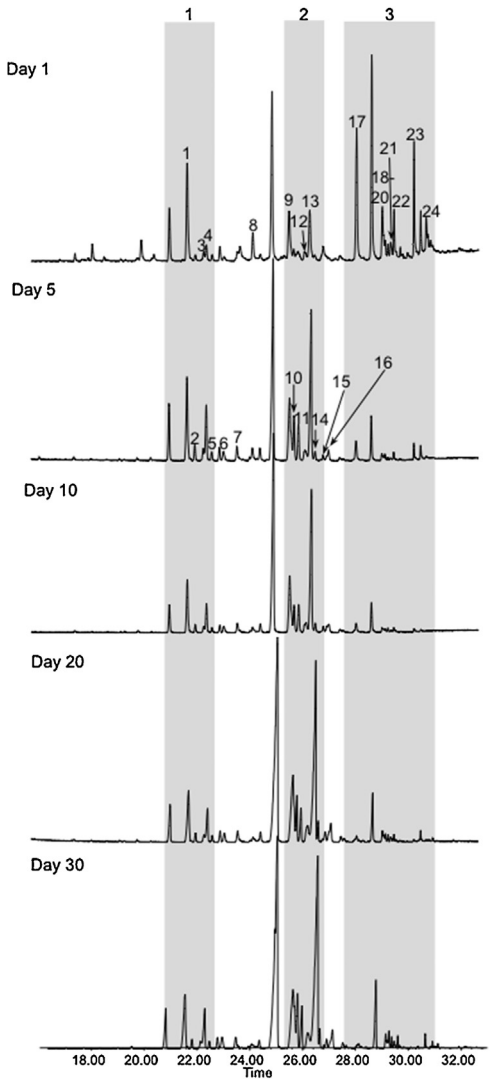

Fig. 1. GC chromatograms of adult female *Cochliomyia macellaria* on day 1, 5, 10, 20 and 30 post-emergence. Shaded bars within the chromatograms illustrate distinctive changes over adult age indicating specific areas of interest.

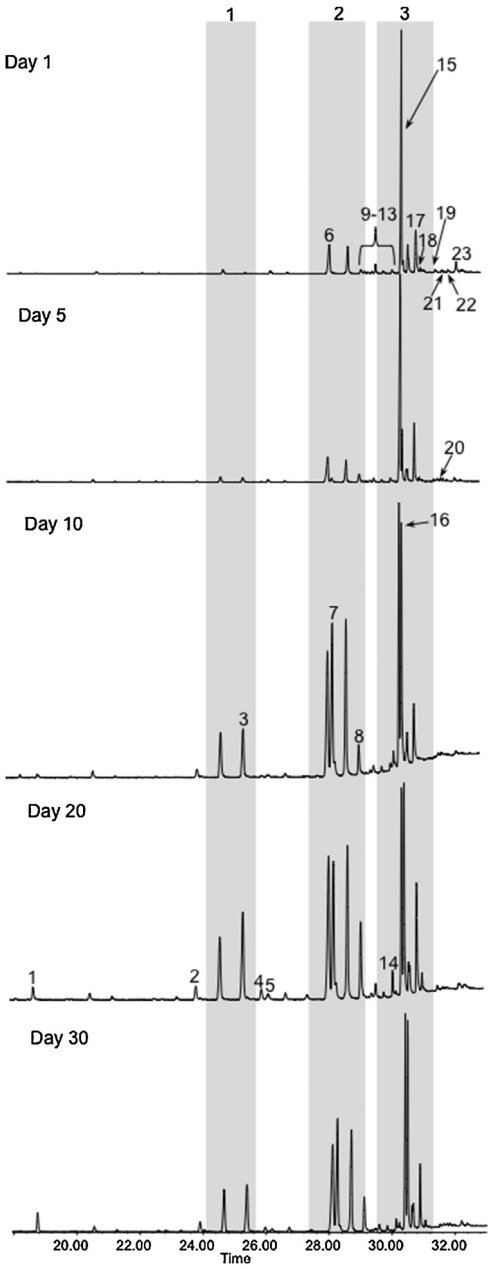

Fig. 2. GC chromatograms of adult female *Chrysomya rufifacies* on day 1, 5, 10, 20 and 30 post-emergence. Shaded bars within the chromatograms illustrate distinctive changes over adult age indicating specific areas of interest.

while day 20 and 30 were represented by increased variability in the profiles, as indicated by the lack of a single clustering for each age cohort. *Chrysomya rufifacies* on the other hand had excellent separation for each day of post-emergence, as seen in Fig. 4, and a two-dimensional NMDS ordination explained 99.5% (stress = 0.041) of hydrocarbon profile variation. The heatmap (Fig. 4) confirmed these distinct age based profile differences with unique clustering occurring on each day post-emergence.

4. Discussion

We identified hydrocarbon compounds specific to *C. macellaria* and *Ch. rufifacies*, with approximately 40% of the same compounds present in both species. The hydrocarbon richness detected in *C. macellaria* increased as the adult female blow flies aged, but reached peak richness on the fifth day post-emergence. This is similar to hydrocarbon profiles detected from *C. hominivorax* (Coquerel) adults; the absolute amount of hydrocarbons, with over 130 hydrocarbon compounds mainly comprised of *n*-alkanes and branched alkanes (85%), increased until flies were five days old [35]. However, this study only analyzed the profiles on newly emerged, three- and five-day old *C. hominivorax* individuals. *Chrysomya rufifacies* profiles on the other hand demonstrated a slight decrease in hydrocarbon richness as the adults aged.

In our study, the hydrocarbon profiles were clearly distinct on the first day post-emergence for both species when compared to any of the other age group (Figs. 3 and 4). There was increased variability in the later days after emergence for both species as indicated by the similar clustering patterns on day 5, 10, 20 and 30 post-emergence. However, *Ch. rufifacies* adults clustered more similarly for day 1 and 5 compared to the remaining days (Fig. 4).

Removal of *n*-alkane compounds from the analyses resulted in distinct profiles and clustering amongst individual days for both taxa, which indicates *n*-alkanes contain the most variation within the profiles of both species. Further, the overall variation in hydrocarbon profiles was reduced when this class of hydrocarbons was excluded from analysis. A decision was made to remove a

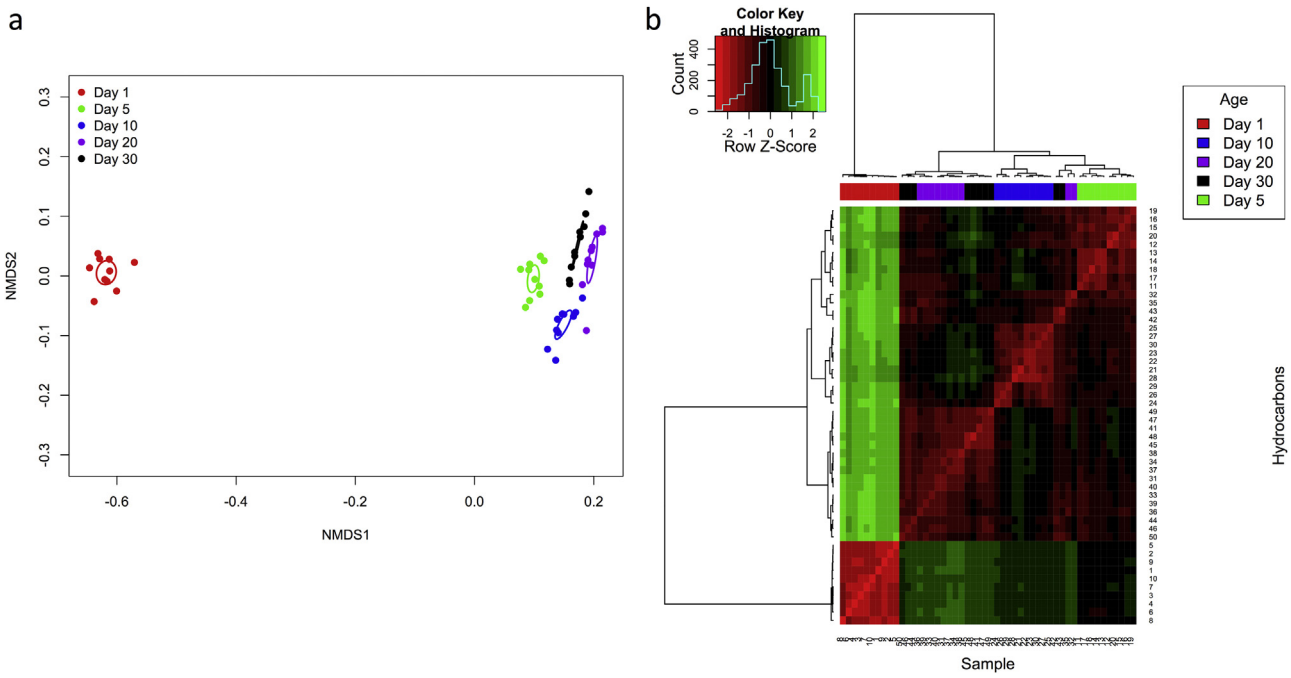

Fig. 3. Bray-Curtis dissimilarity of the on the methyl-branched alkane hydrocarbon profiles of *Cochliomyia macellaria* adult female age on day 1, 5, 10, 20 and 30 were visualized using (A) a non-metric multidimensional scaling ordination plotted on two dimensions (stress = 0.048, R^2 = 0.996); each day has a 95% confidence ellipses around class centroids and (B) a heatmap of the hydrocarbon profiles with the dendrograms clustering by similarities in values on the top and side by the individual samples and hydrocarbon compounds, respectively.

co-eluting peak (2,14-diMethylC30 co-eluting with *n*-C31) as manual integration of the peak could introduce human error into the analysis. Cuticular hydrocarbon studies on ants have shown the *n*-alkanes are a function of environmental protection and vary within the ant profiles of the same nest-mates [36]. This study demonstrated that although *n*-alkanes are abundantly present on the cuticle, they had no role in nest-mate discrimination studies, as the *n*-alkane ratios could vary substantially within individuals from a single colony [37]. This could explain why the discrimination among age cohorts is enhanced when the *n*-alkanes are

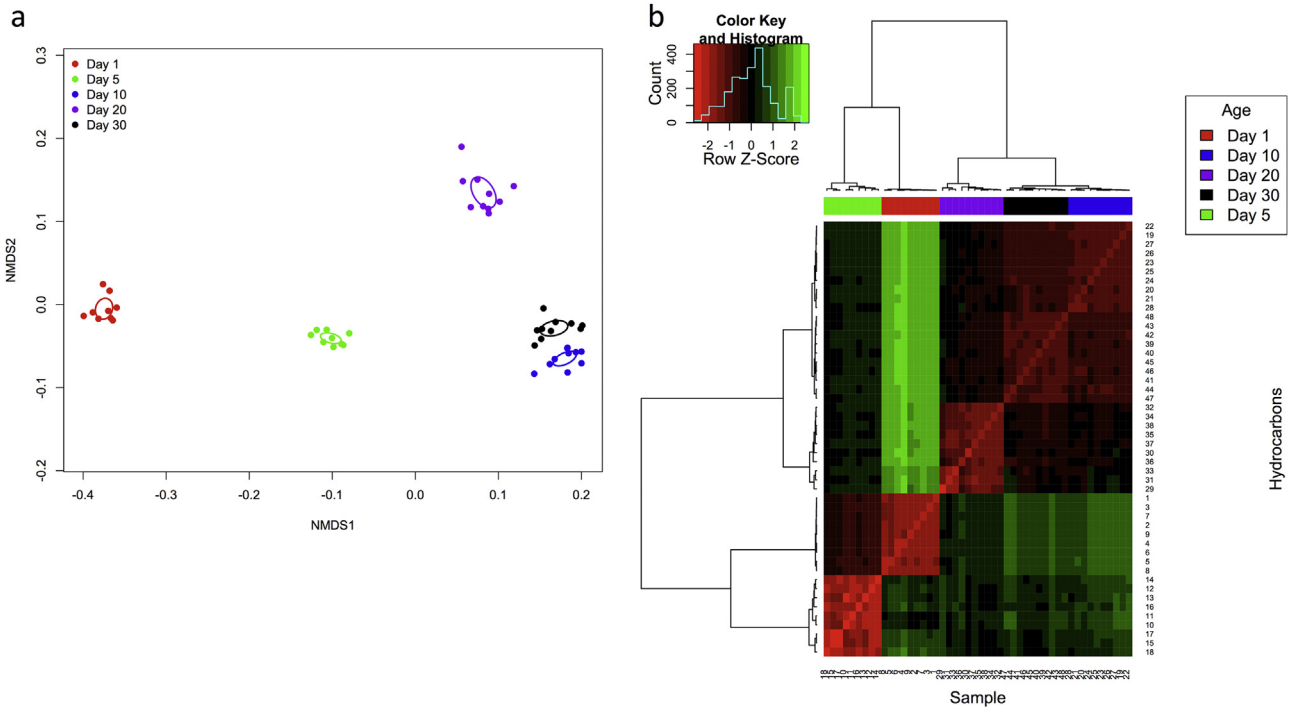

Fig. 4. Bray-Curtis dissimilarity of the on the *n*-alkene and methyl-branched alkane hydrocarbon profiles of *Chrysomya rufifacies* adult female age on day 1, 5, 10, 20 and 30 were visualized using (A) a non-metric multidimensional scaling ordination plotted on two dimensions (stress = 0.041, R^2 = 0.995); each day has a 95% confidence ellipses around class centroids and (B) a heatmap of the hydrocarbon profiles with the dendrograms clustering by similarities in values on the top and side by the individual samples and hydrocarbon compounds, respectively.

removed from the ordinations, as the inherent variation is reduced in the multivariate data structure [38].

The profiles of adult flies were dominated by an abundance of high molecular weight hydrocarbons (with very few low molecular weight compounds present). Adult flies have to withstand much drier environmental conditions, therefore, the cuticle require the larger hydrocarbons which offer increased impermeability under less favorable conditions [18,39]. The hydrocarbon composition changes drastically with age, especially the methyl branched hydrocarbons. Blow fly adults do not sexually mature until approximately 48 h after emergence, depending on the temperature [20]; therefore, it is not surprising that no sex alkenes were detected in the young adult profiles (day 1 post-emergence).

There were unique hydrocarbons profiles (Figs. 1 and 2) detected for the native and invasive blow fly species and as the adults aged within each species. These distinct hydrocarbon compositions can be considered 'fingerprint' chemical profiles, which could potentially be used to distinguish between species and provide age estimates of adults. These results indicate a potential usefulness of this technique for forensic practitioners by: (1) identifying necrophagous insect taxa using non-molecular techniques and (2) estimating the age of an adult insect, thus increasing the importance of collecting adults at a criminal investigation scene. Additionally, these data add to the literature base for the identification of taxa within Calliphoridae using hydrocarbon profile analysis and could be used as a technique in the future to identify morphologically similar taxa such as sister species in *Lucilia* and Sarcophagidae.

Future studies could increase the number of Calliphoridae species (and other forensically important taxa such as beetles) from various geographic regions, which would increase the variability of abiotic conditions in which the individual taxa are exposed to; this could determine if there is a core set of hydrocarbon compounds present across all Calliphoridae taxa regardless of geographic location. Hydrocarbons are known to adapt to temperature and humidity, thus future laboratory based research conducted at variable abiotic conditions is warranted. Finally, Calliphoridae collected from the field experience constant fluctuations in both temperature and humidity, and therefore it is important to assess the effects of these variable abiotic conditions on hydrocarbon profiles in natural populations.

Our data demonstrate unique hydrocarbon profiles for a native, *C. macellaria*, and invasive, *Ch. rufifacies*, blow fly species in North America, and age-related hydrocarbon profiles within each species. This work provides empirical data that serve as a foundation for future research into improving PMI_{min} estimates made by forensic practitioners and potentially increase the use of adult insects during death investigations.

Acknowledgements

The authors would like to thank the Baudendistel family for access to their property and allowing us to decompose swine carcasses on their land, which resulted in the collection of adults to form the Calliphoridae colonies used during this study. The authors would also like to thank J. Alfieri for assistance during the project. This project was funded (HEM) by ACORN from Keele University and The Winston Churchill Memorial Trust. This project was also funded (MEB), in part, by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice through Grant 2010-DN-BX-K243. Points of view in this document are those of the authors and do not necessarily represent the official position or policies of the U.S. Department of Justice.

Appendix A

Table A1.

Table A1
PERMANOVA results testing *Cochliomyia macellaria* and *Chrysomya rufifacies* adult *n*-alkene and methyl-branched alkane hydrocarbon profiles based on Bray-Curtis dissimilarity among 1, 5, 10, 20 and 30 days post-emergence.

Source	d.f.	*SS*	MS	*F*	*P*
Cochliomyia macellaria	4	4.786	1.197	92.123	0.001
Residuals	45	0.584	0.013		
Total	49	5.370			
Chrysomya rufifacies	4	2.042	0.511	183.76	0.001
Residuals	43	0.119	0.003		
Total	47	2.162			

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